



Occurrence of Virus in Some Watermelon Growing Areas of Kwara State, Nigeria

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HIGHLIGHTS

- Viruses associated with watermelon.
- Pathogenic viruses in Ilorin.
- Mixed infections observed.
- CMV is most prevalent.

Abstract

A survey was carried out in September and October 2019 in 8 locations of Kwara State noted for watermelon cultivation to determine the incidence, severity, and distribution of viruses infecting watermelons on the field. Thirty leaf samples showing symptoms like mosaic, chlorosis, stunting, leaf deformation, and necrosis were collected randomly per each farm and analyzed using antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA). Seven viruses were detected across different locations: Cucumber Mosaic Virus (CMV), Watermelon Mosaic Virus (WMV), Cowpea Mottle Virus (CMoV), Cowpea Mild Mosaic Virus (CPMMV), Southern Bean Mosaic Virus (SBMV), Zucchini Yellow Mosaic Virus (ZYMV), and Papaya Ringspot Virus (PRSV). CMV had the highest incidence (100%), followed by CMoV (50%). However, ZYMV and PRSV recorded the lowest incidence with 12.5% and 25%, respectively. The result also revealed that the location with the highest incidence was Agric AJ1 (80%), with the lowest coming from Onireke (35%). The average severity score across the locations was 2.9, with Agric AJ1 and Unilorin having the highest score (4), while Olomu, Eyenkorin, and Lafiaji 1 had the lowest (2). Findings from this research underscore the need to educate farmers on the importance of using resistant cultivars as well as proper cultural practices.

Keywords: Incidence; severity; survey; virus; watermelon

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1. Introduction

Citrullus lanatus (Watermelon) is a member of the family Cucurbitaceae. It is one of the most important fruit crops cultivated across the world for its vegetative and fruit parts, which are highly nutritious (Okunola et al. 2011). Watermelon provides an easy source of vitamins, potassium, iron, and manganese, especially in developing nations of the world where it isn't easy to meet daily nutritional requirements (Dantata 2014). It also contains lycopene, which serves as an antioxidant that neutralizes free radicals. Watermelon rind (outer layer) contains citrulline, which is a non-protein amino acid that helps in blood circulation and heart rate stabilization (Reetu and Tomar 2018). In Sub-Saharan Africa, watermelon is vital to the socioeconomic status of farmers as it serves as a source of income and employment (Enujeke 2013).

Notwithstanding the nutritional and economic benefits of the crop, production in Nigeria has been low as a result of poor adoption of technology, declining soil fertility (Ibrahim et al. 2014), and infection by disease-causing organisms (pathogens). On their part, pathogens cause about 14.1% production loss annually on a global scale by disrupting the function of the crop, resulting in a significant reduction in yield (Agrios 2005).

Plant pathogenic virus alone causes an estimated US\$60 billion yield loss annually worldwide. In watermelons, the symptoms of the virus include mosaic, mottling, necrosis, lesions, and distortion of leaves and other plant parts (Roossinck 2011). This poses a threat to food security. Some of the viruses identified to be causing disease in watermelon include Watermelon mosaic virus (WMV), Zucchini yellow mosaic virus (ZYMV), Alfalfa mosaic virus (AMV), Bean pod mottle virus (BPMV), Papaya ringspot virus-W (PRSV-W), Cucumber mosaic virus (CMV), Squash mosaic virus (SqMV), Melon necrotic spot virus (MNSV), Cucurbit yellow stunting disorder virus (CYSDV), Cucurbit aphid born yellows virus (CABYV), Squash vein yellowing virus (SqVYV), Squash leaf curl virus (SLCuV) and Tobacco ringspot virus (TRSV) (Ali et al. 2012). Nigeria has a population of about 200 million people (the largest in Africa), hence, there is a need to identify those factors that serve as impediments to sustainable food security and satisfy the dietary needs of the population. Unfortunately, watermelon production in Nigeria is about 175.34 metric tons per year, which is hugely less than those in other African regions (Egypt – 1.8 metric tons) as well as those in West Africa (Mali – 551,648, Senegal – 259,225) Sub-region (World Atlas 2019). Nonetheless, there is inadequate information about viruses and other pathogens infecting watermelons in Nigeria.

Therefore, this research aimed to study the viruses that infect watermelon and serve as constraints to the optimization of its production in Kwara state, Nigeria. Kwara State was selected due to its location in the North Central region of Nigeria, and its proximity to Northern and Southern states, necessitating a baseline knowledge of the viruses in the area to enhance production in the state and other Southern states where they are consumed extensively.

2. Materials and Methods

2.1. Area of Study

The experiment was carried out in Kwara State, Nigeria. Kwara covers an estimated area of 32,500km while about 75.3% can be cultivated. The two main climates are the wet and dry seasons. Rainfall spans between 1000 and 1500mm yearly, while the mean temperature ranges between 30 °C and 35 °C. Kwara State falls within the Woodland and tall grass agroecological zone in Nigeria. The state is further subdivided by the Kwara State Agricultural Development Program (ADP) into four ecological zones based on the ecology of the area, cultural beliefs, and ease of administration. The zones are Kaima/Baruteen, which belongs to Zone A, Patigi/Edu, which belongs to Zone B, Moro, Ilorin/Asa, which belongs to Zone C. In contrast, Oke-Ero, Ifelodun, Ekiti, Irepodun, Ifelodun, Isin, and Offa all belong to Zone D (KWADP 2006).

2.2. Sampling Technique

Field sampling was carried out in three agroecological zones of the state: Zone B, C, and D. These zones are the major watermelon-producing areas of Kwara state. The sampling was carried out in September and October 2019, during the cropping season. The criteria used for the selection of the survey area were

availability, accessibility of the farm, and willingness of the farmer to allow sample collection on their farm. The average farm size in each location is not less than 2 acres. All the farms visited intercrop watermelon with other crops: Ajase-Ipo (cucumber, tomato, pepper, cabbage, and maize), Eyenkorin (Palm tree and maize), Unilorin (cucumber), and Lafiagi (tomato). In each location, the Global Positioning System (GPS) coordinate was recorded (Figure 1).

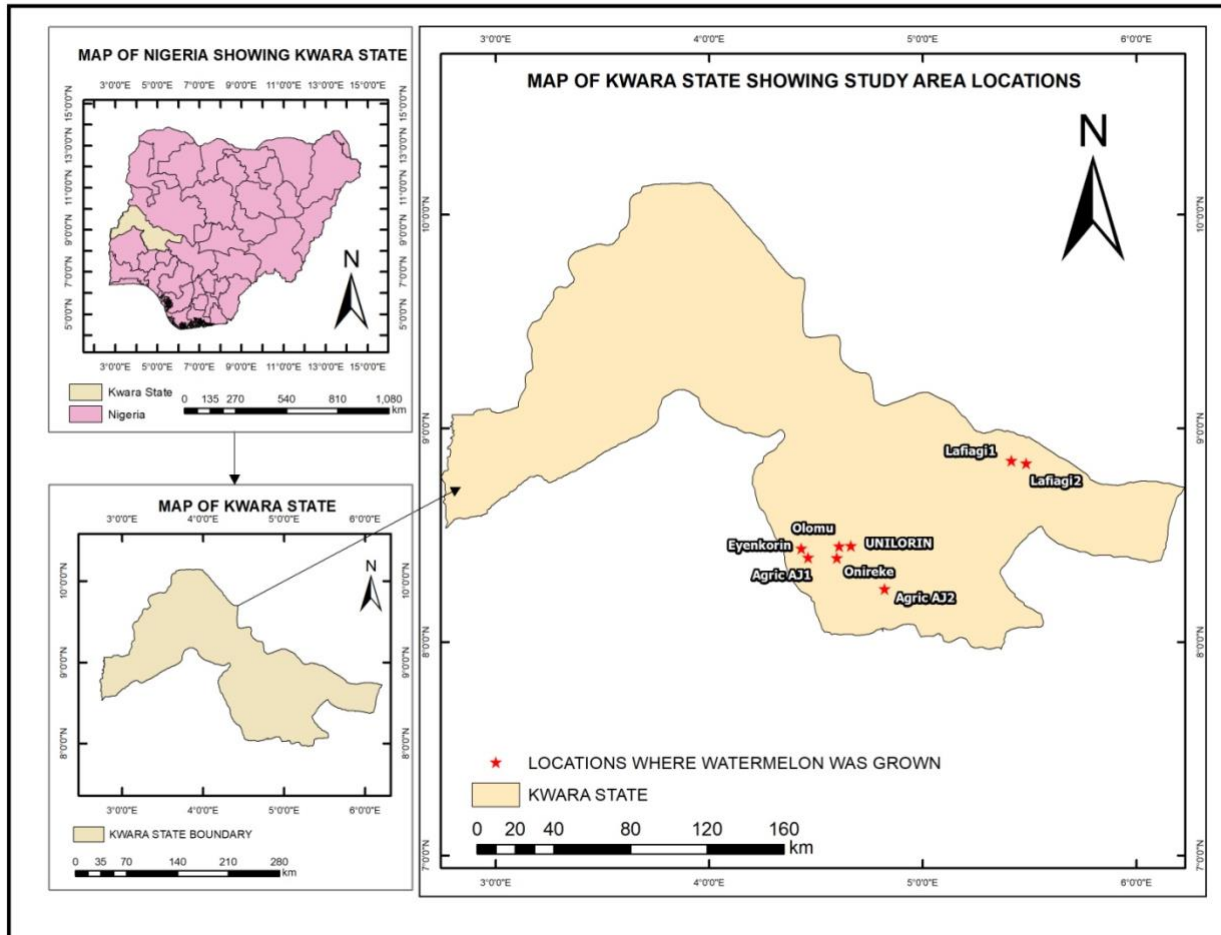


Figure 1. A map showing the locations where the watermelon survey was carried out in Kwara State, Nige

2.3. Virus sampling

Thirty plants per farm were sampled randomly by moving through the field in a “W” shape pattern with five plants each at the same distance from one another (Arogundade et al. 2016). This represents 6 replicates per each area. Young trifoliate leaves showing symptoms of virus infection such as mosaic, chlorosis, stunting, necrosis, and leaf deformation were taken per field (Aliyu 2014). Each of the sampled leaves was kept inside a vial with a silica gel and cotton wool before virus identification in the laboratory. Part of the samples was used for ELISA in other to identify viruses present while the remaining samples were kept for molecular study of the most prominent virus in the areas sampled (Aliyu et al. 2013).

2.4. Estimation of virus incidence and severity rating

The percentage incidence of the virus in each location is determined by using the following formula;

$$\text{Percentage incidence} = \frac{\text{Number of infected plants}}{\text{Number of plants sampled (30)}} \times 100, \quad (1)$$

The severity of infection was estimated based on the percentage number of leaves showing symptoms of the virus out of 30 plants observed on a farm. The samples were scored on a scale of 1-5 using a modified method by Eni et al. (2009);

1 = 1 to 20% (exceedingly mild), 2 = 21 to 40% (mild), 3 = 41 to 60% (severe), 4 = 61 to 80% (extremely severe). 5 = 81 to 100% (nearly dead)

The mean of the scores was used to ascertain the severity of each location. The Kruskal-Wallis test was used to determine the differences in seriousness across the area. In contrast, the Chi-square test was used to examine the relationship between the severity of each location. The software, Statistical Package for Social Sciences (SPSS) was used for the analysis.

2.5. Identification of virus using Enzyme-linked immunosorbent assay (ELISA)

The Antigen-coated plate (ACP) – ELISA method was used in detecting the viruses associated with watermelon: Watermelon mosaic virus (WMV), Cucumber mosaic virus (CMV), Papaya ringspot virus-W (PRSV-W), Zucchini yellow mosaic virus (ZYMV), Cowpea Mottle Virus (CMoV), Cowpea Mild Mosaic Virus (CPMMV), Southern Bean Mosaic Virus (SBMV), Zucchini Yellow Mosaic Virus (ZYMV), and Papaya Ringspot Virus (PRSV). The homologous rabbit polyclonal antisera were used to test the leaves.

Coating ELISA plates with antibodies: CMV polyclonal antiserum was diluted to 1:10,000 in carbonate coating buffer and dispensed to 100 µl into an individual well inside ELISA plate. Then, the ELISA plate was wrapped and transferred into a humid chamber for one hour at 37°C. It was washed with three changes of Phosphate-Buffered Saline-Tween (PBS-T) and allowed 3 minutes at each wash.

2.6. Preparation of leaf extract and virus detection

Symptomatic leaf sample of 0.1 g was ground inside a buffer of carbonate coating (0.015 M Na₂CO₃ and 0.0349 M NaHCO₃) of 1 ml. Then, 100 µL was dispensed into each well of the antibody-coated ELISA plates and incubated in a humid chamber for one hour at 37 °C. The plate was washed with three changes of PBS-T (comprising 0.05% (v/v) and Tween-20), allowing 3 min per wash. The Polyclonal antisera were cross-adsorbed using uninfected watermelon extract from the leaf (1:20 w/v) and diluted in a buffer that is conjugated (PBS-T had 0.02% (w/v) egg albumin and 0.2% (w/v) PVP-40,000). The plates were kept in a humid chamber and incubated at 37 °C for one hour. Then, plates were washed with three changes of PBS-T, allowing 3 min for each wash.

So, rabbit antibody of alkaline phosphatase was employed as a secondary antibody at the rate of 100 µl and 1:15000 (v/v) dilutions. It was kept for one hour at 37 °C. PBS was used three times while 100 µl p-nitrophenyl phosphate (0.001 g/ml) in 10% (v/v) diethanolamine buffer (pH 9.8) was added to each well of the ELISA plate and incubated for one hour. The healthy watermelon plant was used as a negative control. The measurement of absorbance was done after one hour at 405 nm in a BIO-RAD multiscan ELISA reader. Each sample was counted as positive only when the ELISA reading was twice that of a healthy watermelon (control). In case of a positive reaction, the colorless substrate will change to a light yellow, then a deep yellow colour.

3. Results

The serological analysis showed the viruses associated with watermelon and the locations where they were detected in Kwara State (Table 1, and Figure 2). Seven viruses were detected across all the areas sampled which are: Cucumber mosaic virus (CMV), Watermelon Mosaic Virus (WMV), Cowpea Mottle Virus (CMeV), Cowpea Mild Mosaic Virus (CPMMV), Southern Bean Mosaic Virus (SBMV), Zucchini Yellow Mosaic Virus (ZYMV), Papaya Ringspot Virus (PRV). Results also revealed that Cucumber Mosaic Virus had the highest prevalence which was detected in all locations (100%). Meanwhile, Zucchini Yellow Mosaic Virus (ZYMV) was only detected in Unilorin but absent in other locations (12.5%).

Table 1. The distribution of viruses detected on watermelon in Kwara state, Nigeria

S/n	Area	Location	Cucumber mosaic virus	Watermelon Mosaic Virus (WMV)	Cowpea Mottle Virus (CMoV)	Cowpea Mild Mosaic Virus (CPMMV)	Southern Bean Mosaic Virus (SBMV)	Zucchini Yellow Mosaic Virus (ZYMV)	Papaya Ringspot Virus (PRSV)
1	Olomu	8°26'16"N 4°26'1"E	+	+	+	-	+	-	+
2	Eyenkorin	8°23'38"N 4°27'55"E	+	+	+	-	+	-	+
3	Agric AJ1	8°23'33"N 4°35'55"E	+	+	-	+	-	-	-
4	Agric AJ2	8°14'47"N 4°49'19"E	+	-	+	+	-	-	-
5	Unilorin	8°26'57"N 4°39'52"E	+	-	+	-	+	+	-
6	Onireke	8°26'52"N 4°36'36"E	+	-	-	-	-	-	-
7	Lafiaji 1	8°50'56"N 5°24'58"E	+	-	-	-	-	-	-
8	Lafiaji 2	8°51'10"N 5°24'59"E	+	-	-	-	-	-	-

Note: Positive = +, Negative = -

Agric AJ= Agricultural settlement Ajase-ipo

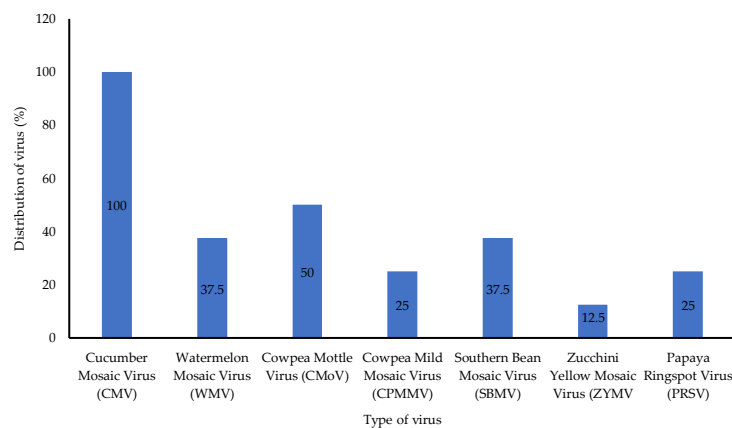

Figure 2. The percentage distribution of different viruses infecting different watermelon-in Kwara State, Nigeria

Table 2 showed that locations visited have a severity score that ranges from mild to very severe (2 to 4). The highest severity score (4) was detected in Agric AJ2 and Unilorin, while the least severe (2) locations were Olomu, Eyenkorin, and Lafiaji_1. The remaining locations were rated 'severe' (3). The average score across different locations was 2.9.

Table 2. Severity score from watermelon growing areas of Kwara State

S/n	Area	Severity score
1	Olomu	2
2	Eyenkorin	2
3	Agric AJ1	3
4	Agric AJ2	4
5	Unilorin	4
6	Onireke	3
7	Lafiaji1	2
8	Lafiaji2	3

The result of the Kruskal-Wallis test to determine the severity rank differences in the severity of locations sampled is shown in Table 3. Agric AJ2 and Unilorin had the highest rank while Olomu, Eyenkorin and Lafiaji 1 had the lowest. Result also showed there was no significant difference in the severity scores across the group.

Figure 3 showed that the Percentage incidence was highest in Agric AJ1 (80%), which Agric AJ2 followed with a 78% percentage. Olomu was next with an incidence of 70%, while Eyenkorin followed with a percentage incidence of 63%. Unilorin followed this with a 63% incidence rate. Lafiaji 2 was next with 52% incidence while Lafiaji 1 had an incidence of 48%. Onireke had the lowest percentage incidence of 35%.

Table 3. Kruskal-Wallis test to rank the severity score in each area and determine their differences

Mean rank of severity score		Test statistics	
Olomu	2	Chi-square	7
Eyenkorin	2	df	7
Agric AJ1	5	Asymptotic Significance	0.42
Agric AJ2	7.5		
Unilorin	7.5		
Onireke	5		
Laiagi1	2		
Lafiaji2	5		

Figure 4 shows that Zone C had the highest percentage incidence of 79%. This was followed by Zone D with a percentage incidence of 66.5%. Zone B had the least incidence, with an average percentage of 50%.

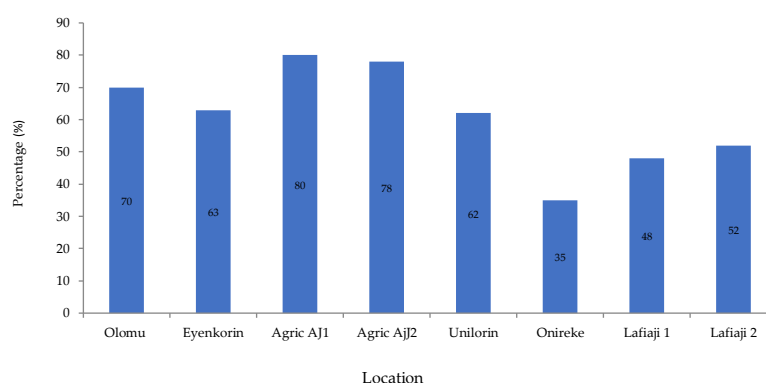


Figure 3. The percentage incidence of virus on watermelon from different locations in Kwara State

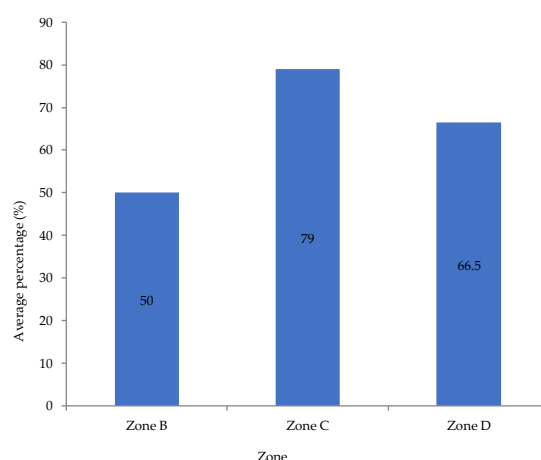


Figure 4. The percentage incidence of virus in three watermelon-producing zones of Kwara state, Nigeria

4. Discussion

This investigation revealed that infection of watermelon on the field is mixed rather than a single infection (Table 1). For instance, three or more infections were observed in Olomu, Eyenkorin, Unilorin, Agric AJ1, and Agric AJ2. Multiple-virus infections are a common phenomenon in the field that could further increase plant susceptibility and symptom severity. This could have been the reason samples taken from the surveyed area have severe symptom that ranges from mosaic, yellowing, mottling, shoestring, etc which are diagnostic symptoms of different viruses (Ziebell et al. 2011). Adediji et al. (2020), reported similar results when they observed co-infections of a potyvirus and several other viruses on tomato and pepper hosts. This also agrees with the observation of Raimundo et al. (2015), when they reported mixed infections of viruses in watermelons on the field in Tocantins, Brazil.

CMV had 100% distribution in all the locations which makes it the most prevalent virus across all the area surveyed (Figure 2). This was followed by CMeV with 50% of the locations, WMV and SBMV with 37.5%, CPMMV, and PRV with 25%, and ZYMV with 12.5%. The high distribution of CMV implies that the rate of transmitting the virus is high in those areas. Since there is no evidence of vertical transmission of CMV in watermelon, one major factor to be considered in this case is the possibility that vectors of the virus may be prevalent in those areas. Although the vector population was not sampled, nevertheless, there is a high probability of increased incidence of their vectors. Inadequate cultural practices could be another reason for the prevalence of CMV in those areas. Crops with similar host ranges were noticeably cultivated close to watermelon, which could have served as an alternate host to the virus. For instance, cucumber, tomato, pepper, cabbage, and maize were planted around the area where the watermelon was planted. These crops are natural hosts of CMV (Pratap et al. 2012), and planting them close to each other could have increased their chances of transmission from one plant to another. CMV has been reported to have the most extensive range among plant viruses (Roossinck 2001), and is particularly pathogenic to cucurbits in both temperate and tropical regions (Ali et al. 2012; Milojevic et al. 2012). This could also be another reason incidence was highest in Zone C which had 79% infection (Figure 4). Notably, intercropping watermelon with other cucurbits was most common in Agric AJ1, Agric AJ2, Olomu, and Eyenkorin which are in the same zone and were also the areas with the highest incidence of the disease (Figure 3). This study also corroborates the report of Arogundade et al (2015), that the proximity of pepper to other host plants of CMV contributed greatly to a high incidence of the virus. Mechanical transmission could also be another reason for the high rate of CMV infection. Unlike many viruses, CMV is fairly stable and can be sap-transmitted by farmers when infected tools and clothing are used during farm operations like weeding, transplanting, and thinning (Akhtar et al. 2008). Notwithstanding, the severity score was not significantly different across the group which suggests that the differences in severity across locations may be due to random variation or possibly the plant genotype planted in those areas. A previous study by Kimani et al. (2024), suggested that plant genotype has effect on the transmission and epidemiology of virus diseases. This study necessitates the need to educate farmers more on disease management practices.

5. Conclusions

This study revealed the susceptibility of watermelon to different viruses as well as the wide spread of the viruses in different areas of the state. The occurrence of symptoms in all the locations indicates the susceptibility of the varieties cultivated in the study area. Though different viruses were detected in this study, however, there are more other viruses that were not tested. Therefore, more tests should be conducted to determine viruses and other pathogens that limit watermelon production. In order to attain sustainable food production, there is a need to also up-scale extension services to educate farmers on proper cultural practices in plant disease management.

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Conflicts of Interest: We, the authors, declare that there is no conflict of interest.

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